

# Anti-DNA activity in systemic lupus erythematosus

## A diagnostic and therapeutic guide

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Several autoantibodies have been associated with systemic lupus erythematosus (SLE). Attention has been directed in particular to antibodies reactive with deoxyribonucleic acid (DNA) (Seligmann, 1957; Robbins, Holman, Deicher, and Kunkel, 1957; Stollar, Levine, and Marmur, 1962; Tan, Schur, Carr, and Kunkel, 1966) because the antigen involved is well defined and because anti-DNA antibodies are relatively specific for SLE. In addition there is evidence that DNA-anti-DNA complexes may play a role in the pathogenesis of nephritis complicating SLE (Christian, 1969; Koffler, Schur, and Kunkel, 1967; Andres, Accini, Beiser, Christian, Cinotti, Erlanger, Hsu, and Seegal, 1971).

It is apparent that many anti-DNA antibodies exist (Arana and Seligmann, 1967; Cohen, Hughes, Noel, and Christian, 1970) and that techniques such as complement fixation and gel precipitation, commonly used in their study, each detect limited subpopulations of antibodies. The ammonium sulphate precipitation technique (Farr, 1958; Wold, Young, Tan, and Farr, 1968) is a sensitive method for the detection of anti-DNA antibodies independent of their biological properties. It promises to be of value in the study and management of patients with SLE (Pincus, Schur, Rose, Decker, and Talal, 1969).

In the present study, anti-DNA activity, measured both by agarose precipitation and by the Farr technique, was correlated with the clinical activity and course of SLE.

### Material and methods

Sera were stored at  $-20^{\circ}\text{C}$ . Double diffusion was carried out in agarose gel (0.7 per cent. in pH 7.6 veronal) using both native (double stranded) and heat denatured (single stranded) salmon sperm DNA (Worthington Biochemical

Laboratories). The DNA was denatured by heating at  $100^{\circ}\text{C}$ . for 10 minutes, followed by rapid chilling. Both native and denatured DNA were diluted in Tris buffer (pH 7.4) to 0.1 mg./ml.

Serum complement levels were measured by the method of Kent, Bukantz, and Rein (1946). Normal levels range from 150 to 210  $\text{CH}_{50}$  units.

### Ammonium sulphate precipitation technique (Fig. 1)

The test is based on the insolubility of immune complexes in 50 per cent. saturated ammonium sulphate. To 5  $\mu\text{l}$ . serum, diluted to 50  $\mu\text{l}$ . in borate saline buffer (0.1 M NaCl) (pH 8), 0.1  $\mu\text{g}$ .  $^{14}\text{C}$ -labelled native DNA in 50  $\mu\text{l}$ . borate buffer was added. (The  $^{14}\text{C}$ -DNA, derived from KB cells grown in a medium containing  $^{14}\text{C}$  thymidine, was kindly supplied by Dr. T. Pincus). After overnight incubation at  $4^{\circ}\text{C}$ ., 100  $\mu\text{l}$ . chilled saturated ammonium sulphate was added. After centrifugation, 100  $\mu\text{l}$ . supernate was removed and its radioactivity and that of the remaining 100  $\mu\text{l}$ . containing the

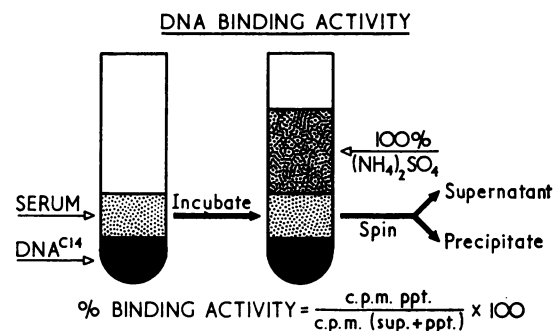


FIG. 1 FARR TECHNIQUE.  $^{14}\text{C}$ -labelled DNA is added to the test serum. DNA-anti-DNA complexes, if formed, are precipitated on addition of 100 per cent. saturated ammonium sulphate. The amount of radioactivity in the precipitate portion thus measures the anti-DNA antibody activity of the serum.

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precipitate were determined by liquid scintillation spectrometry. 'Per cent. binding activity' was calculated from the formula:

$$\frac{\text{Precipitate C.P.M.} - \text{Supernatant C.P.M.}}{\text{Precipitate C.P.M.} + \text{Supernatant C.P.M.}} \times 100$$

Results

(1) AGAROSE PRECIPITATION

21 patients with precipitating anti-DNA antibodies were studied. These sera could be divided into two groups (Table): those that reacted with both native and heat denatured DNA, and those that reacted with denatured DNA alone. The immunological differences between these two groups have been presented elsewhere (Cohen and others, 1971). Precipitating antibodies rapidly disappeared with therapy and clinical improvement (Fig. 2). Patients with the most severe renal disease, as judged by

clinical criteria, renal function tests, and renal biopsy gave the strongest precipitin lines with native DNA, and complement levels tended to be lower in this group.

(2) FARR TECHNIQUE

The diagnostic value of the Farr technique for measurement of anti-DNA antibodies in SLE is illustrated in Fig. 3. Normal sera bound less than 20 per cent. of the labelled DNA. In all but two sera from patients with diseases other than SLE, binding activity was below 20 per cent. (These two patients were initially diagnosed as cases of juvenile rheumatoid arthritis, but they subsequently manifested features characteristic of SLE). In 185 serum samples from 86 SLE patients, values ranged from 10 to 100 per cent. No patient with clinical evidence of active disease had a DNA-binding value less than 20 per cent.

Table Particulars of 21 patients with precipitating antibodies

Patient	Serum complement	DNA-binding (per cent.)	Precipitating antibody	
			Native	Denatured
L.S.	< 50	100	+	+
S.P.	< 50	100	+	+
D.M.		100	+	+
D.W.	127	100	+	+
J.M.	< 50	100	+	+
E.O.	—	100	+	+
L.G.	61	100	+	+
E.B.	< 50	100	+	+
I.S.	—	100	+	+
G.B.	146	100	+	+
S.M.	156	100	+	+
F.N.	< 50	100	+	+
W.R.	< 50	100	+	+
M.T.	< 50	100	+	+
V.G.	—	100	+	+
N.A.	106	100	—	+
B.S.	77	100	—	+
N.H.	127	100	—	+
N.J.	111	100	—	+
Q.D.	112	78	—	+
K.M.	78	100	—	+
I.G.	80	100	—	+

Note: All except one had 100 per cent. DNA binding as measured by the Farr technique (see later). In the first six patients, a stronger reaction with native DNA was obtained. Sera below the line gave precipitin reactions with denatured DNA only.

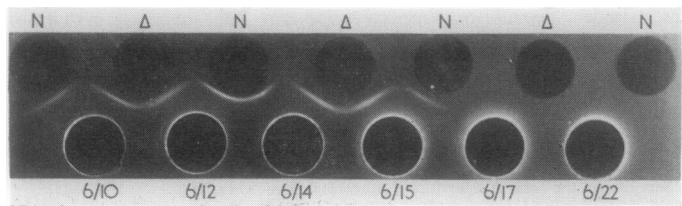


FIG. 2 Lower wells contain serum taken on successive dates from Patient E.O. Corticosteroid therapy, started on June 15 resulted in the disappearance of precipitating antibodies to both native DNA (N) and denatured DNA (Δ) within 48 hrs of treatment.

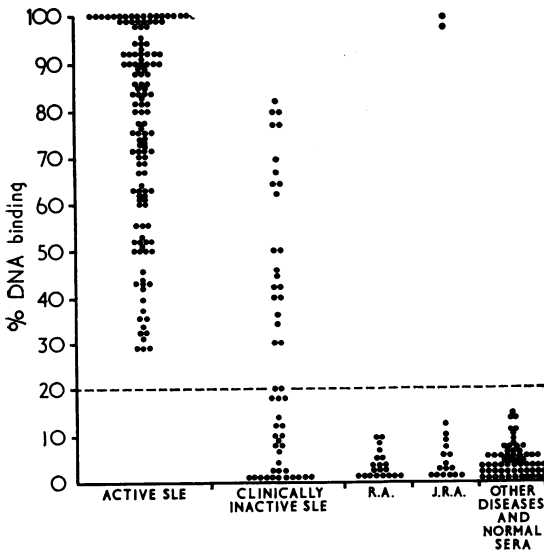
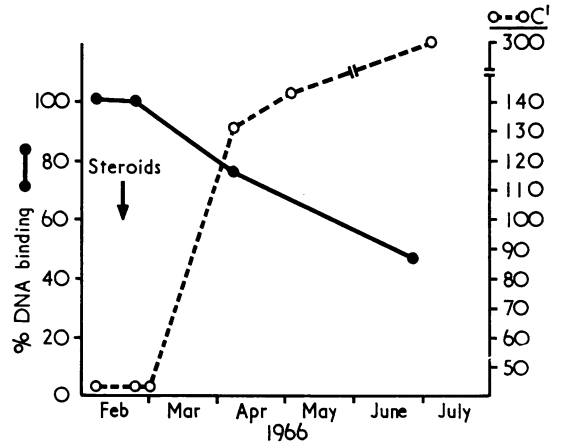


FIG. 3 Scattergram showing per cent. DNA-binding activity in 185 serum samples from 86 SLE patients (divided into clinically active and inactive groups), twenty cases of rheumatoid arthritis, eighteen cases of juvenile rheumatoid arthritis, 39 patients with other diseases, including, drug-induced lupus, and 24 normal subjects.

In all but one of the 21 sera with precipitating anti-DNA antibodies, DNA-binding activity was 100 per cent. Normal values were obtained in patients with sero-positive RA (20 cases), juvenile rheumatoid arthritis (18 cases), dermatomyositis (5 cases), progressive systemic sclerosis (6 cases), Reiter's syndrome (4 cases), drug-induced lupus erythematosus (11 cases). Many of the sera in these groups had been found to have antinuclear antibodies, as measured by immunofluorescence.

To illustrate correlations between serological events (anti-DNA antibodies and serum complement levels) and variables in the clinical manifestations related to therapy, the experience with six patients studied for different periods of time is summarized below:

**Case 1, a 35-year-old Negro female with active SLE (E.B.),** showed both clinical and serological improvement after the institution of corticosteroid therapy (Fig. 4). At the onset of this study, when the disease was clinically active, serum complement levels were below 50  $\text{CH}_{50}$  units and DNA-binding activity was 100 per cent. Therapy with prednisone (60 mg./day) resulted in clinical improvement. The serum complement level increased and the DNA-binding activity decreased. However, 4 months after starting therapy, at a time when symptoms were absent and the serum complement level was in the range of normal, the DNA-binding activity was still 48 per cent. This patient subsequently died of *Klebsiella pneumoniae* and bacteraemia.



FIGS 4-8 Serial studies of patients with SLE, showing changes in serum complement and anti-DNA antibody titre with treatment and clinical events.

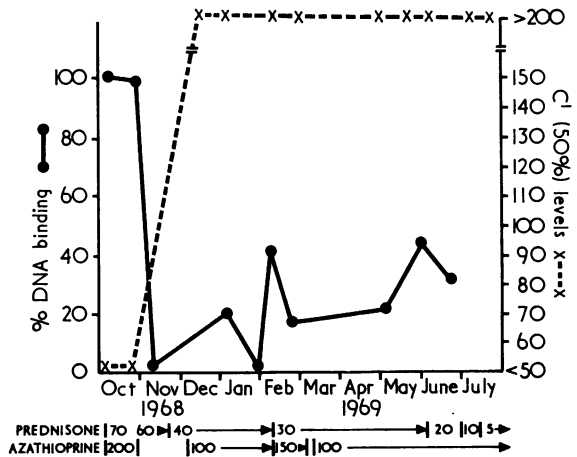


FIG. 5

**Case 2, a female patient (L.S.),** presented with acute nephritis and a serum complement level below 50  $\text{CH}_{50}$  units (Fig. 5). Her serum contained precipitating antibodies to both native and denatured DNA. After starting prednisone (70 mg./day) and azathioprine (200 mg./day), she showed clinical and serological improvement. DNA-binding activity fell to normal levels within 2 weeks.

During the following 6 months, she remained free from clinical evidence of SLE activity, and the serum complement levels remained normal, but the DNA-binding activity rose. In January, 1970, she again developed clinical signs of activity with polyarthritis and a skin rash requiring increased corticosteroid therapy. She has since been symptom-free on prednisone 10 mg./day and azathioprine 200 mg./day.

**Case 3, a 30-year-old Puerto Rican male (W.R.; Fig. 6)** with SLE, was followed over the period of one year.

In January, 1969, when he was first seen in this institution, his disease activity was characterized by fever, nephritis, and myositis, with histological evidence of vasculitis, and many LE-cells in the peripheral blood. The serum complement level was less than 50 and

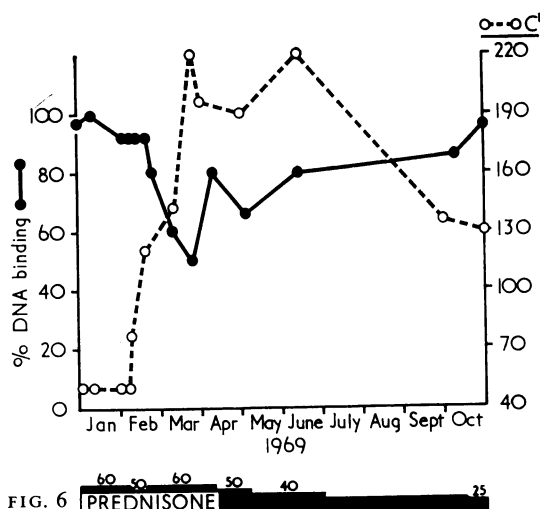


FIG. 6

DNA-binding activity 100 per cent. On prednisone (60 mg./day) the patient improved, his serum complement rose, DNA-binding activity fell, and precipitating anti-DNA antibodies disappeared.

2 months later, the patient was well, the serum complement had returned to normal levels, and precipitating anti-DNA antibodies were absent, but DNA-binding activity remained high (80 per cent.). During the ensuing months the DNA-binding activity fell to 50 per cent. and began to rise again, reaching 80 per cent. by October, 1969. Throughout this period the patient remained well on decreasing doses of prednisone.

In November, 1969, the complement level again fell below normal. A month later the patient developed an exacerbation of disease, with severe polyarthritis and gross haematuria which responded to an increase in corticosteroid therapy.

**Case 4, a 15-year-old girl** (P.B.: Fig. 7) with SLE, had been in remission and without therapy for 2 years after an episode of nephritis. While still in remission, she was found to have DNA-binding activity of 80 per cent. and a serum complement level of 85. Routine renal function tests (BUN, electrolytes, urine analysis) were normal apart from a trace of proteinuria. This case illustrates that high DNA-binding activity may herald the onset of a clinical relapse, for 4 weeks later, she became grossly nephrotic. With prednisone therapy, the clinical and serological abnormalities reverted to normal and the remission of serological and clinical abnormalities has been maintained on prednisone 15 mg./day and azathioprine 150 mg./day (not shown in diagram).

In one patient, serological abnormalities led to a suspicion of renal involvement despite normal renal function tests.

**Case 5, a 22-year-old white woman**, gave a 3-months history of polyarthritis and chills. She was found to have a malar eruption, widespread synovitis, fever, lymphopenia, and LE cells. Creatinine clearance, BUN, IVP, and daily urine analyses were normal. She was found to

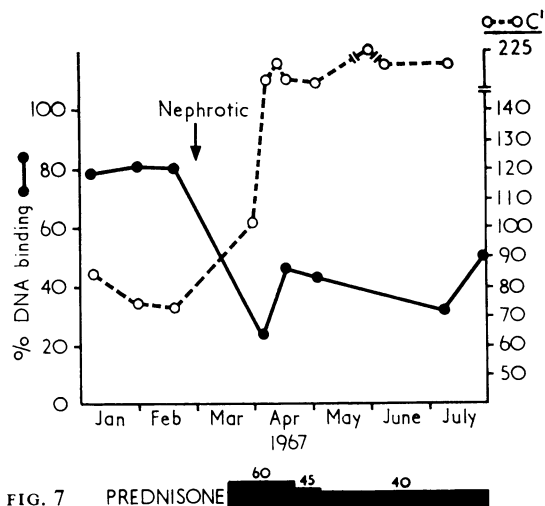


FIG. 7

have DNA-binding activity of 100 per cent., serum complement less than 50 units, and precipitating antibodies to both native and denatured DNA. Because of these immunological abnormalities a percutaneous renal biopsy was performed. Widespread focal proliferative changes, affecting all glomeruli taken, were seen on light microscopy. Immunofluorescent examination\* revealed mesangial deposits of IgM and complement.

On prednisone 60 mg./day, the complement and anti-DNA antibody titres slowly returned towards normal during the following 3 months. Repeated urine analyses remained normal over this period.

**Case 6 (Fig. 8)** In one SLE patient the approximate inverse relationship between DNA-binding activity and complement levels was not observed. In November, 1968, the patient developed thrombocytopenic purpura, the platelet count falling to 12,000 per cu. mm. There were no other clinical signs of SLE activity and apart from prednisone (15 mg./day) the patient was not taking any drug before this episode. During this hypocomplement-

\*Kindly performed by Dr. David Koffler, The Rockefeller University, New York, N.Y.

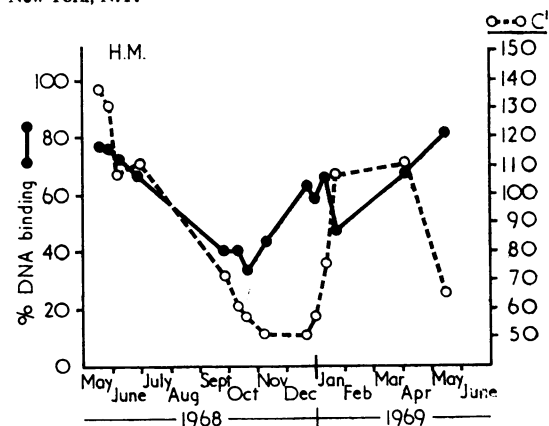


FIG. 8

aemic period the DNA-binding activity also fell, though not to normal levels. Throughout the episode the patient remained otherwise well and has remained symptom-free on prednisone 5 to 10 mg./day.

## Discussion

The significance of antibodies to DNA in patients with SLE has been well established. Methods used to detect such antibodies in the past have included precipitation, complement fixation, immunofluorescence, passive haemagglutination, and ammonium sulphate precipitation techniques.

It is apparent that the Farr ammonium sulphate precipitation procedure, using  $^{14}\text{C}$ -labelled native DNA, is a highly sensitive test that correlates with the clinical activity of SLE. The gel diffusion method for detecting precipitating antibodies is a simple, but much less sensitive, assessment of anti-DNA activity.

While precipitation in agarose appears to require more than one interacting subpopulation of anti-DNA antibodies, it is probable that the ammonium sulphate precipitation technique detects all antibodies to native DNA. For this reason, the spectrum of SLE activity in which anti-DNA activity was detectable by this method was wide, normal binding values being found only in quiescent cases. Many sera with high DNA-binding values gave negative complement-fixation and precipitin reactions for anti-DNA antibodies. The presence of precipitating antibodies is usually a temporary phenomenon in the face of adequate therapy, the antibodies often disappearing within a few days of initiation of corticosteroid therapy. This rapid disappearance cannot be explained by decreased synthesis alone and may reflect rapid removal of anti-DNA antibodies as immune complexes, possible with DNA released during suppressive therapy (Hughes, Cohen, Lightfoot, Meltzer, and Christian, 1971).

Despite the high sensitivity of the test, specificity for SLE was noted also. This lack of 'false positive' results may simply be a reflection of the relatively small numbers tested to date. Pincus and others (1969) noted abnormal DNA-binding activity in six out of 24 cases of Sjögren's syndrome tested, and three of these patients have subsequently developed overt SLE (Pincus, 1969). The present series of patients included only six with documented Sjögren's syndrome, none of whom showed anti-DNA activity.

Because two patients initially diagnosed as having juvenile rheumatoid arthritis had high DNA-binding activity, a total of eighteen patients with this diagnosis were studied—no other cases with high anti-DNA activity were detected and the two patients in question subsequently manifested a multi-system disease compatible with SLE.

The test may prove valuable in differentiating

SLE from other systemic diseases, especially those in which the immunofluorescent test for anti-nuclear antibodies is often positive. In RA and scleroderma, including those patients in whom antinuclear antibodies had been detected, anti-DNA antibodies were absent, and in eleven cases of 'drug-induced lupus' (ten by Procainamide and one by Isoniazid) normal values were obtained.

The serial studies of SLE patients led to a number of observations. While there was, in general, a close correlation of DNA-binding with clinical activity and an appropriate inverse relationship of complement levels, exceptions were seen. After the institution of therapy, complement levels often returned to normal before anti-DNA activity (For example, W.R. in Fig. 6).

Anti-DNA activity may provide a guide to adequacy of therapy in SLE. In Case 2 both clinical improvement and the fall in anti-DNA activity were brisk, despite the severity of the disease.

Perhaps more important is the possibility that a rising or persistently high DNA-binding level may herald an exacerbation of disease. In this situation, abnormal DNA-binding activity may precede a falling complement level, and both in turn may precede clinical worsening (W.R.: Fig. 6).

It is not known whether treatment during periods of remission of SLE affects the ultimate prognosis. Because of the weight of evidence implicating immune complexes, including DNA-anti-DNA complexes, in the pathogenesis of SLE nephritis, high anti-DNA activity in the presence of a low serum complement level may be an indication for instituting or increasing suppressive therapy.

Schur and Sandson (1968) noted that, in SLE, very low complement levels and high titres of antibodies to DNA (measured by complement fixation in their study) were always associated with active renal disease and (Koffler, Carr, Agnello, Fiezi, and Kunkel, 1969), using haemagglutination procedures, found that antibodies to native DNA occurred almost exclusively in serums of patients in the active stages of SLE.

The decision to place a totally asymptomatic patient on pharmacological doses of corticosteroids or of an immunosuppressive drug because of serological abnormalities is difficult. Current experience suggest that such a course is indicated for the patient with low complement and anti-DNA antibodies if previous episodes of active nephritis have correlated with these phenomena. The indications for aggressive treatment are not clear when a minimally symptomatic subject without current or past life-threatening manifestations of SLE presents with decreased serum complement and/or anti-DNA antibodies. Although low serum complement levels in SLE probably reflect the presence of circulating immune complexes,

which conceptually are deleterious, recent data suggest that some SLE patients may have complement depressions on the basis of decreased synthesis (Sliwinski and Zvaifler, 1970).

In the light of present knowledge, however, a reversion to normal of serum complement and anti-DNA antibody titre on high-dose steroid therapy suggests that these serological criteria can subsequently be used as guides in the management in that patient.

### Summary

Antibodies to native DNA have been found highly specific for SLE. With the introduction of the ammonium sulphate (globulin) precipitation technique, a sensitive method for measuring these antibodies has become available.

In this paper, the diagnostic significance of antibodies to native DNA and their possible value

in management and prognosis have been explored, using both gel diffusion and globulin precipitation techniques.

Under the conditions of study, all sera from patients with active SLE had DNA-binding levels in excess of 20 per cent., and in all normal subjects and controls with other diseases the levels were lower than 20 per cent. The test, because of its sensitivity in measuring anti-DNA antibodies, appears to be a more useful diagnostic and therapeutic guide to SLE than other tests at present available.

The clinical and serological events in representative cases have been summarized, illustrating the general correlation of such antibodies and decreased levels of serum complement with activity of disease. Since the above serological phenomena may antedate clinical exacerbations of SLE nephritis, they can provide guides in management, and may lead to better control of this disease.

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